(12) UK Patent Application (19) GB (11) 2 236 185 (13) A

(43) Date of A publication 27.03.1991

(21) Application No 9018334.4

(22) Date of filing 21.08.1990

(30) Priority data
(31) 3927602 (32) 22.08.1989 (33) DE
3937165 08.11.1989
4017805 01.06.1990

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(51) INT CL[5]
G01N 37/00

(52) UK CL (Edition K)
G1B BAX BCH

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(58) Field of search
UK CL (Edition K) G1B BAX BCH
INT CL[5] G01N 37/00
Online databases: WPI, DIALOG/BIOTECH

(54) Laser desorbtion of analyte bound on a surface

(57) A process for making an analyte available in which the analyte molecules are bound in an essentially two-dimensional layer on a surface of a specimen comprises desorption of the analyte molecules from the surface via laser desorption.

A specimen for carrying out the said process comprises components which are able to absorb laser light and a device for carrying out the process comprises a laser whose laser light can be swept over the zones of the specimen.

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Fig.1

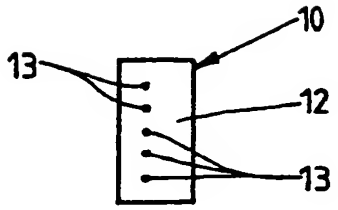


Fig. 2

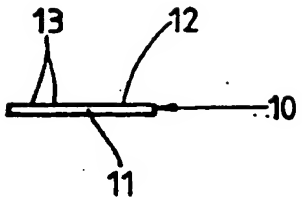


Fig. 3

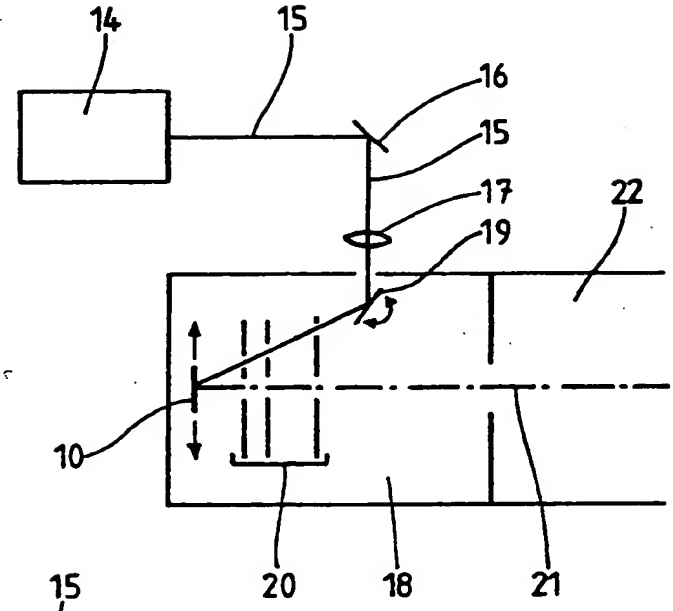
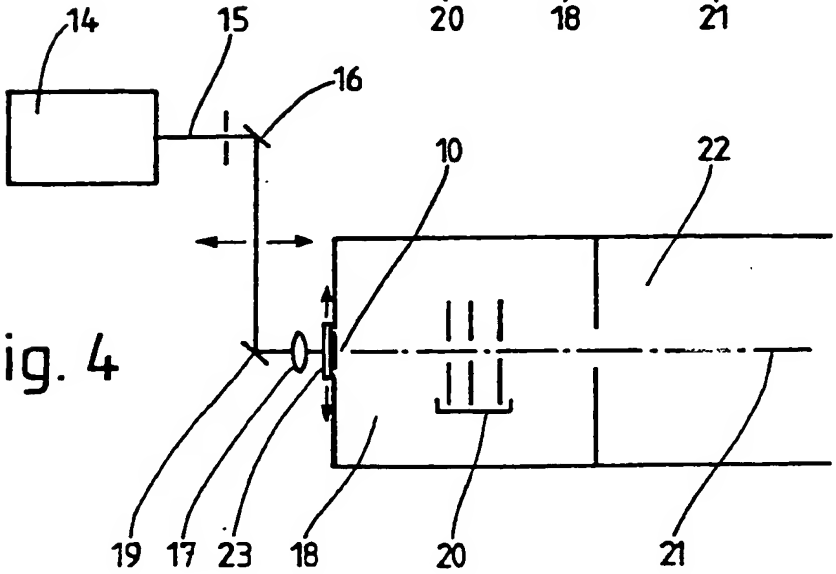


Fig. 4



Process, specimen and device for making an analyte available for an investigation

Description

The invention relates to a process for making an analyte available for an investigation, in which the analyte molecules are bound in an essentially two-dimensional layer on a surface of a specimen.

The invention furthermore relates to a specimen for use in the abovementioned process and a device for carrying out the process.

In many practical problems which are posed, an analyte which is to be (further) investigated is essentially in the form of a two-dimensional monolayer, and generally also immobilized, on a surface, or it is applied in this form to a surface which is suitable or to be made suitable. Examples of this are one- or two-dimensional electrophoresis gel plates, chromatography plates or sensor surfaces of biosensors.

The problem which arises for an investigation or further investigation, for example after chromatography or electrophoresis, of the analyte is that the analyte molecules must be specifically removed from the surface, for example for an investigation by mass spectrometry. In general terms, it is necessary to make the analyte molecules available for an investigation or further investigation.

Hence the object of the invention is to improve the process mentioned in the introduction for making an analyte available for an investigation.

The object is achieved according to the invention by desorbing the analyte molecules from the surface by laser desorption.

This process step according to the invention can be used to remove analyte molecules in an advantageous manner specifically from a surface and to make them available for an investigation, for example also by mass spectrometry.

Laser desorption, especially for preparation for

mass spectrometry is known per se for three-dimensional samples in which the analyte molecules are present in a volume distribution in a suitable matrix.

Laser desorption is now advantageously used according to the invention also for making an analyte available from a surface for an investigation. It is surprising that laser desorption can also be used for essentially two-dimensional surface samples.

The laser desorption according to the invention is made possible by use of a specimen which consists of the analyte and components for absorbing the laser energy. An example of a suitable absorbing component is nicotinic acid. However, other suitable examples are thymine, pyrazinecarboxylic acid, thiourea or vanillic acid.

The substance which is chosen as substrate on whose surface the analyte is bound can be one which itself is suitable for absorbing laser light, for example polycarbonate.

An essentially two-dimensional sample can be transferred (blotted) onto another surface for the (further) investigation, in which case the assignment of individual analyte zones is retained on the second surface. An example of a suitable substrate for surface blotting is nitrocellulose.

The analyte molecules can be bound via spacer molecules on the specimen surface, in which case the spacer molecules themselves can be absorbers of laser light, for example L-3,5-dinitrobenzoylphenylglycine, or it is possible to use spacer molecules which do not absorb laser light, for example propylamine.

The analyte molecules can be subjected to chemical reactions by means of chemical reagents before the laser desorption. For example, typical reagents for breaking down proteins are proteases, for example trypsin.

The substrate of the specimen can be chosen such that a chromatographical or electrophoretic separation of analytes in an analyte mixture can be carried out. A

suitable separating substrate would be the polyacrylamide which is normally used in electrophoresis.

If separate zones of analyte molecules are present on a surface, it is possible to apply laser desorption successively to these zones by scanning laser light over these zones in succession. It is possible for this purpose to move a laser and the specimen relative to one another.

The device according to the invention for carrying out the laser desorption is distinguished by a laser which transmits laser light to a specimen, it preferably being possible to scan zones of the specimen with the laser light.

A specimen which is used in the process according to the invention is preferably distinguished by a substrate on whose surface analyte molecules are bound. The specimen has components for absorbing laser light. In this connection, the specimen can be the original sample itself, but the specimen can also be a blot of the original sample.

Exemplary embodiments from which further inventive features emerge are depicted in the drawing. The diagrams show in:

Fig. 1 a plan view of a specimen with which analyte molecules are made available for an investigation,

Fig. 2 a side view of the specimen shown in Fig. 1,

Fig. 3 a plan view of a device for carrying out a laser desorption for making analyte molecules available for an investigation according to a first exemplary embodiment of the invention and

Fig. 4 a plan view of a device for carrying out a laser desorption for making analyte molecules available for an investigation according to a second exemplary embodiment of the invention.

Fig. 1 shows a plan view of a specimen 10 which consists of a backing plate 11 (Fig. 2) and of a substrate layer 12 applied to the backing plate 11. Individual analyte zones 13 are indicated on the surface of

the substrate layer 12. In these analyte zones 13, analyte molecules are bound essentially two-dimensionally on the surface of the substrate 12. The different analyte zones 13 indicated in Fig. 1 might, for example, be derived from a single analyte zone of a mixture of analytes from which the analytes have been separated by chromatography. In this chromatography the distances migrated by the analytes in the individual analyte zones 13 on the substrate surface differ.

Apart from the analytes, the substrate layer 12 also has components which can absorb laser light. Components of this type can also be the substrate components themselves. It is possible in this way for the analytes in the specimen shown in Fig. 1 to be made available by laser desorption for an investigation or further investigation.

The specimen 10 shown in Fig. 1 can be an original sample, but it can also be a blot of an original sample, the analyte zones 13 having been transferred, while retaining their mutual correlation, from a surface onto the surface of the substrate layer 12 of the specimen 10.

Fig. 2 shows a side view of the specimen 10 shown in Fig. 1, from which it can be seen that the analyte zones 13 are present in an essentially two-dimensional layer.

Fig. 3 shows a plan view of an exemplary embodiment for a device with which analyte molecules in a specimen 10 can be made available by means of laser desorption for an investigation.

The device shown in Fig. 3 comprises a laser 14 which emits a laser beam 15. The laser beam 15 is deflected by means of a first reflecting mirror 16 and focused by means of a focusing lens 17. After the focusing, the laser beam 15 enters the vacuum chamber 18 of an ion source. A specimen 10, which can be, for example, attached to a specimen support, is located in this vacuum chamber 18 of the ion source. The laser beam 15 entering the vacuum chamber 18 is deflected a second time by a

second reflecting mirror 19. After this, the laser beam 15 impinges on the specimen 10.

In order for it to be possible to scan the individual analyte zones 13 of the specimen 10 with the laser beam 15, the specimen 10 is arranged to be displaceable in its specimen plane and/or the second deflecting mirror 19 is rotatably mounted, as is indicated in each case by arrows in Fig. 3.

An ion-optical system 20 of the ion source is located in the vacuum chamber 18 of the ion source and is used to extract, and focus to an ion beam 21, the analyte molecules or analyte ions which are removed by laser desorption from the specimen 10. The ion beam 21 emerges from the vacuum chamber 18 of the ion source into a vacuum chamber 22 of an analyzer with which the analyte molecules are investigated. The analyzer can be, for example, a mass spectrometer.

The device shown in Fig. 4 comprises a laser 14 which emits a laser beam 15. The laser beam 15 is directed by means of two reflecting mirrors 16 and 19 onto a transparent sample support 23, and focused via the focusing lens 17 into the plane of the specimen 10. The sample support 23 is transparent for the laser wavelength used and simultaneously serves for vacuum sealing of the vacuum chamber 18. The specimen 10 is located, as depicted in Fig. 2, on the side facing the analyzer.

The laser beam 15 can be scanned with the aid of the reflecting mirrors 16 and 19 over the analyte zone, and where the extent of the sample is relatively large it is possible to displace the sample support relative to the laser beam, as is indicated by corresponding arrows in Fig. 4.

An ion-optical system 20 of the ion source is again located in the vacuum chamber 18 of the ion source and is used to extract, and to focus to an ion beam 21, the analyte molecules or analyte ions which are removed by laser desorption from the specimen 10. The ion beam 21 emerges from the vacuum chamber 18 of the ion source into a vacuum chamber 22 of an analyzer with which the analyte

molecules are investigated. The analyzer can be, for example, a mass spectrometer.

Claims:

1. Process for making an analyte available for an investigation, in which the analyte molecules are bound in an essentially two-dimensional layer on a surface of a specimen, characterized in that the analyte molecules are dissorbed from the surface by laser desorption.

2. Process according to Claim 1, characterized in that, besides the analyte, the specimen is also mixed with components for absorbing the laser energy as constituents.

3. Process according to Claim 2, characterized in that nicotinic acid is added as absorbing component.

4. Process according to one of the preceding claims, characterized in that the substance chosen as substrate on whose surface the analyte is bound is itself suitable for absorbing laser light.

5. Process according to Claim 4, characterized in that polycarbonate is used as substrate.

6. Process according to one of Claims 1 to 3, characterized in that the absorbing components are applied before (underneath) the analyte.

7. Process according to one of Claims 1 to 3, characterized in that the absorbing components are applied after (on top of) the analyte.

8. Process according to Claim 6 or 7, characterized in that the absorbing components are applied by spraying, centrifugation or vacuum deposition.

9. Process according to one of the preceding claims, characterized in that analyte zones are transferred (blotted), while retaining their correlation to one another, from another support down onto the specimen surface.

10. Process according to Claim 9,characterized in that nitrocellulose is used as substrate for the specimen, onto which transer (blotting) is carried out.

11. Process according to one of the preceding claims, characterized in that the analyte molecules are bound via spacer molecules on the specimen surface.

12. Process according to Claim 11, characterized in

that propylamine is used as spacer.

13. Process according to Claim 11, characterized in that the spacer molecules chosen are suitable for absorbing laser light.

14. Process according to Claim 13, characterized in that L-3,5-dinitrobenzoylphenylglycine is used as spacer.

15. Process according to one of the preceding claims, characterized in that the analyte molecules are subjected to chemical reactions by means of chemical reagents before the laser desorption.

16. Process according to Claim 15, characterized in that a protease is used as chemical reagent for breaking down proteins.

17. Process according to one of the preceding claims, characterized in that the binding of the analyte molecules to the surface is loosened or destroyed shortly before the laser desorption.

18. Process according to one of the preceding claims, characterized in that the substance chosen as substrate on whose surface the analyte molecules are bound is one which is suitable for carrying out a chromatographical or electrophoretic separation of a mixture of various analytes, and that a separation of this type is carried out before the laser desorption.

19. Process according to Claim 18, characterized in that polyacrylamide is used as substrate for carrying out an electrophoresis.

20. Process according to Claim 18 or 19, characterized in that the analyte zones of different analyte molecules which have been separated from one another by chromatography or electrophoresis are successively scanned and irradiated with laser light using a laser.

21. Process according to Claim 20, characterized in that the specimen and the laser beam are moved relative to one another for the scanning.

22. Specimen which is used in the process according to one of the preceding claims and in which analyte molecules are bound in an essentially two-dimensional layer on a surface of a substrate, characterized by

components which are able to absorb laser light.

23. Specimen according to Claim 22, characterized in that zones (13) with analyte molecules have been transferred (blotted) onto the specimen surface from another sample surface.

24. Specimen according to Claim 23, characterized in that the substrate of the specimen onto whose surface the analyte zones (13) are transferred (blotted) is nitrocellulose.

25. Specimen according to one of Claims 22 to 24, characterized by spacer molecules which bind the analyte molecules on the specimen surface.

26. Specimen according to one of Claims 22 to 25, characterized in that the substrate of the specimen is suitable for carrying out a chromatographic or electrophoretic separation of a mixture of various analytes.

27. Specimen according to Claim 26, characterized in that the substrate is polyacrylamide.

28. Device for making an analyte available, the analyte molecules of which are bound in an essentially two-dimensional layer, preferably in various zones, on a surface of a specimen, characterized by a laser (14) for carrying out a laser desorption, the laser beam (15) and the specimen (10) being movable relative to one another for scanning the specimen surface with the laser beam (15).

29. Device according to Claim 28, characterized in that the laser beam (15) is arranged to be movable in a defined manner.

30. Device according to Claim 28 or 29, characterized in that the specimen (10) is arranged to be movable in a defined manner.

31. Device according to one of Claims 28 to 30, characterized in that at least one guiding or deflecting element (19) for guiding or deflecting the laser beam (15) is arranged to be movable in such a way that the laser beam (15) can be swept over the analyte zones (13) of the specimen (10).

32. Device according to one of Claims 28 gto 31,

characterized by a time and location control for the laser scanning.

33. A device for making an analyte available, substantially as hereinbefore described with reference to figures 3 and 4 of the accompanying drawings.

34. A specimen substantially as hereinbefore described with reference to figures 1 and 2 of the accompanying drawings.

35. A process substantially as hereinbefore described with reference to the accompanying drawings.

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